Amendments to the Specification:

The paragraph starting at page 1, line 16 has been amended as follows:

--There is a large body of literature on the actions and activities of IGFs (IGF-1, IGF-2, and IGF variants). Human IGF-1 is a serum protein of 70 amino acids and 7649 daltons with a pI of 8.4 (Rinderknecht and Humbel, Proc. Natl. Acad. Sci. USA, 73: ~~2365~~ 4379-4381 (1976); Rinderknecht and Humbel, J. Biol. Chem., 253: 2769 (1978)) belonging to a family of somatomedins with insulin-like and mitogenic biological activities that modulate the action of growth hormone (GH) (Van Wyk *et al.*, Recent Prog. Horm. Res., 30: 259 (1974); Binoux, Ann. Endocrinol., 41: 157 (1980); Clemmons and Van Wyk, Handbook Exp. Pharmacol., 57: 161 (1981); Baxter, Adv. Clin. Chem., 25: 49 (1986); U.S. Pat. No. 4,988,675; WO 91/03253; WO 93/23071). IGFs share a high sequence identity with insulin, being about 49% identical thereto. Unlike insulin, however, which is synthesized as a precursor protein containing a 33-amino-acid segment known as the C-peptide (which is excised to yield a covalently linked dimer of the remaining A and B chains), IGFs are single polypeptides (see Figure 1). --

The paragraph starting at page 1, line 39 has been amended as follows:

-- IGFs have been implicated in a variety of cellular functions and disease processes, including cell cycle progression, proliferation, differentiation, and insulin-like effects in insulin-resistant diabetes. Thus, IGF has been suggested as a therapeutic tool in a variety of diseases and injuries ~~(for review, see Lowe, Scientific American (March/April 1996), p. 62)~~. Due to this range of activities, IGF-1 has been tested in mammals for such widely disparate uses as wound healing, treatment of kidney disorders, treatment of diabetes, reversal of whole-body catabolic states such as AIDS-related wasting, treatment of heart conditions such as congestive heart failure, and treatment of neurological disorders (Guler *et al.*, Proc. Natl. Acad. Sci. USA, 85: 4889-4893 (1988);

Schalch *et al.*, J. Clin. Metab., 77: 1563-1568 (1993); Froesch *et al.*, Horm. Res., 42: 66-71 (1994); Vlachopapadopoulou *et al.*, J. Clin. Endo. Metab., 12: 3715-3723 (1995); Saad *et al.*, Diabetologia, 37: Abstract 40 (1994); Schoenle *et al.*, Diabetologia, 34: 675-679 (1991); Morrow *et al.*, Diabetes, 42 (Suppl.): 269 (1993) (abstract); Kuzuya *et al.*, Diabetes, 42: 696-705 (1993); Schalch *et al.*, "Short-term metabolic effects of recombinant human insulin-like growth factor I (rhIGF-I) in type II diabetes mellitus", in: Spencer EM, ed., Modern Concepts of Insulin-like Growth Factors (New York: Elsevier: 1991) pp. 705-~~715~~713 ; Zenobi *et al.*, J. Clin. Invest., 90: 2234-2241 (~~1993~~1992); Elahi *et al.*, "Hemodynamic and metabolic responses to human insulin-like growth factor-1 (IGF-I) in men," in: Modern Concepts of Insulin-Like Growth Factors, Spencer, EM, ed. (Elsevier: New York, 1991), pp. 219-224; ~~Quinn~~ Quin *et al.*, New Engl. J. Med., 323: 1425-1426 (1990); Schalch *et al.*, "Short-term metabolic effects of recombinant human insulin-like growth factor 1 (rhIGF-I) in type II diabetes mellitus," in: Modern Concepts of Insulin-Like Growth Factors, Spencer, EM, ed., (Elsevier: New York, 1991), pp. 705-~~714~~713; Schoenle *et al.*, Diabetologia, 34: 675-679 (1991); Usala *et al.*, N. Eng. J. Med., 327: 853-857 (1992); Lieberman *et al.*, J. Clin. Endo. Metab., 75: 30-36 (1992); Zenobi *et al.*, J. Clin. Invest., 90: 2234-2241 (1992); Zenobi *et al.*, J. Clin. Invest., 89: 1908-1913 (1992); Kerr *et al.*, J. Clin. Invest., 91: 141-147 (1993); Jabri *et al.*, Diabetes, 43: 369-374 (1994); Duerr *et al.*, J. Clin. Invest., 95: 619-627 (1995); Bondy, Ann Intern. Med., 120: 593-601 (1994); Hammerman and Miller, Am. J. Physiol., 265: F1-F14 (1993); Hammerman and Miller, J. Am. Soc. Nephrol., 5: 1-11 (1994); and Barinaga *et al.*, Science, 264: 772-774 (1994)).--

The paragraph starting a page 3, line 7 has been amended as follows:

--IGF-1R is a key factor in normal cell growth and development (Isaksson *et al.*, Endocrine Reviews, 8: 426-438 (1987); Daughaday and Rotwein, Endocrine Rev., 10:68-91 (1989)). Increasing evidence suggests, however, that IGF-lR signaling also plays a

critical role in growth of tumor cells, cell transformation, and tumorigenesis (Baserga, Cancer Res., 55:249-252 (1995)). Key examples include loss of metastatic phenotype of murine carcinoma cells by treatment with antisense RNA to the IGF-lR (Long *et al.*, Cancer Res., 55:1006-1009 (1995)) and the *in vitro* inhibition of human melanoma cell motility (Stracke *et al.*, J Biol. Chem., 264:~~21554-21559~~ 21544-21549 (1989)) and of human breast cancer cell growth by the addition of IGF-1R antibodies (Rohlik *et al.*, Biochem. Biophys. Res. Commun., 149:276- 281 (1987)). --

The paragraph starting at page 4, line 12 has been amended as follows:

-- In most cases, addition of exogenous IGFBP blunts the effects of IGF-l. For example, the growth- stimulating effect of estradiol on the MCF-7 human breast cancer cells is associated with decreased IGFBP-3 mRNA and protein accumulation, while the anti-estrogen ICI 182780 causes growth inhibition and increased IGFBP-3 mRNA and protein levels (Huynh *et al.*, J Biol. Chem., 271:1016-1021 (1996); Oh *et al.*, Prog. Growth Factor Res., 6:503-512 (1995)). It has also been reported that the *in vitro* inhibition of breast cancer cell proliferation by retinoic acid may involve altered IGFBP secretion by tumor cells or decreased circulating IGF-1 levels *in vivo* (LeRoith *et al.*, Ann. Int. Med., 122:54-59 (1995); Oh *et al.*, (1995), *supra*). Contrary to this finding, treatment of MCF-7 cells with the anti-estrogen tamoxifen decreases IGF-lR signaling in a manner that is unrelated to decreased IGFBP production (Lee *et al.*, J Endocrinol., 152:39 (1997)). Additional support for the general anti-proliferative effects of the IGFBPs is the striking finding that IGFBP-3 is a target gene of the tumor suppressor, p53 (Buckbinder *et al.*, Nature, 377:646-649 (1995)). This suggests that the suppressor activity of p53 is, in part, mediated by IGFBP-3 production and the consequential blockade of IGF action (Buckbinder *et al.*, *supra*). These results indicate that the IGFBPs can block cell proliferation by modulating paracrine/autocrine processes regulated by IGF-1/IGF-2. A corollary to these observations is the finding that prostate-specific

antigen (PSA) is an IGFBP-3-protease, which upon activation, increases the sensitivity of tumor cells to the actions of IGF-1/IGF-2 due to the proteolytic inactivation of IGFBP-3 (Cohen *et al.,* J. Endocr., 142:407-415 (1994)). The IGFBPs complex with IGF-1/IGF-2 and interfere with the access of IGF- l/IGF-2 to IGF-lRs (Clemmons *et al.*, Anal. NY Acad. Sci. USA, 692:10-21 (1993)). IGFBP-1, -2 and -3 inhibit cell growth following addition to cells *in vitro* (Lee *et al.* , J Endocrinol., 152:39 (1997); Feyen *et al.*, J Biol. Chem., 266:19469-19474 (1991)). Further, IGFBP-1 (McGuire *et al.*, J Natl. Cancer Inst., 84:~~1335~~1336-1341(1992); Figueroa *et al.*, J Cell Physiol., 157:229-236 (1993)), IGFBP-3 (Oh *et al.* (1995), *supra*; Pratt and Pollak, Biophys. Res. Commun., 198:292-297 (1994)) and IGFBP-2 have all been shown to inhibit IGF-1 or estrogen-induced breast cancer cell proliferation at nanomolar concentrations *in vitro*. These findings support the idea that the IGFBPs are potent antagonists of IGF action. There is also evidence for a direct effect of IGFBP-3 on cells through its own cell surface receptor, independent of IGF interactions (Oh *et al.*, J Biol. Chem., 268:14964-14971 (1993); Valentinis *et al.*, Mol. Endocrinol., 9:361-367 (1995)). Taken together, these findings underscore the importance of IGF and IGF-lR as targets for therapeutic use.--

The disclosure between page 5, line 37 and page 6, line 32 has been amended as follows:

--Also reported was an IGF variant that binds to IGFBPs but not to IGF receptors and therefore shows reduced activity in *in vitro* activity assays (Bar *et al.*, Endocrinology, 127: 3243-3245 (1990)). In this variant, designated (1-27,gly^4, 38-70)-hIGF-1, residues 28-37 of the C-region of human IGF-1 (SEQ ID NO: 1) are replaced by a four-residue glycine bridge.

Other truncated IGF-1 variants are disclosed. For example, in the patent literature, WO 96/33216 describes a truncated variant having residues 1-69 of authentic IGF-1 (SEQ ID NO: 1). EP 742,228 discloses two-chain IGF-1 superagonists, which are derivatives of the naturally occurring, single-chain IGF-1 having an abbreviated C-region. The IGF-

1 analogs are of the formula: BC^n,A wherein B is the B-region of IGF-1 or a functional analog thereof, C is the C-region of IGF-1 (SEQ ID NO: 1) or a functional analog thereof, n is the number of amino acids in the C-region and is from about 6 to about 12, and A is the A-region of IGF-1 or a functional analog thereof.

Additionally, Cascieri *et al.*, Biochemistry, 27: 3229-3233 (1988) discloses four mutants of IGF-1 (SEQ ID NO: 1), three of which have reduced affinity to IGF-1R. These mutants are: (Phe^{23},Phe^{24},Tyr^{25})IGF-1 (which is equipotent to human IGF-1 in its affinity to the Types 1 and 2 IGF and insulin receptors), (Leu^{24})IGF-1 and (Ser^{24})IGF-1 (which have a lower affinity than IGF-1 to the human placental IGF-1R, the placental insulin receptor, and the IGF-1R of rat and mouse cells), and desoctapeptide (Leu^{24})IGF-1 (in which the loss of aromaticity at position 24 is combined with the deletion of the carboxyl-terminal D-region of hIGF-1 (SEQ ID NO: 1), which has lower affinity than (Leu^{24})IGF-1 for the IGF-1R and higher affinity for the insulin receptor). These four mutants have normal affinities for human serum binding proteins.

Bayne *et al.*, J. Biol. Chem., 263: 6233-6239 (1988) discloses four structural analogs of human IGF-1 (SEQ ID NO: 1): a B-chain mutant in which the first 16 amino acids of IGF-1 were replaced with the first 17 amino acids of the B-chain of insulin, (Gln^3,Ala^4)IGF-1, (Tyr^{15},Leu^{16})IGF-1, and (Gln^3,Ala^4,Tyr^{15},Leu^{16})IGF-1. These studies identify some of the regions of IGF-1 that are responsible for maintaining high-affinity binding with the serum binding protein and the Type 2 IGF receptor.

In another study, Bayne *et al.*, J. Biol. Chem., 264: 11004-11008 (1988) discloses three structural analogs of IGF-1 (SEQ ID NO: 1): (1-62)IGF-1, which lacks the carboxyl-terminal 8-amino-acid D-region of IGF-1; (1-27,Gly^4,38-70)IGF-1, in which residues 28-37 of the C-region of IGF-1 are replaced by a four-residue glycine bridge; and (1-27,Gly^4,38-62)IGF-1, with a C-region glycine replacement and a D-region deletion. Peterkofsky *et al.*, Endocrinology, 128: 1769-1779 (1991) discloses data using the Gly^4 mutant of Bayne *et al.*, *supra* (vol. 264).

Cascieri *et al.*, J. Biol. Chem., 264: 2199-2202 (1989) discloses three IGF-1 analogs in which specific residues in the A-region of IGF-1 (SEQ ID NO: 1) are replaced with the corresponding residues in the A chain of insulin. The analogs are: (Ile^{41},Glu^{45},Gln^{46},Thr^{49},Ser^{50},Ile^{51},Ser^{53},Tyr^{55},Gln^{56})IGF-1, an A-chain mutant in which residue 41 is changed from threonine to isoleucine and residues 42-56 of the A-region are replaced; (Thr^{49},Ser^{50},Ile^{51})IGF-1; and (Tyr^{55},Gln^{56})IGF-1.

The paragraph starting at page 7, line 18 has been amended as follows:

--Additionally, ~~EP 639981~~ WO 93/23067 discloses pharmaceutical compositions comprising short peptides that function as IGF-1 receptor antagonists. The peptides used in the pharmaceutical compositions consist of less than 25 amino acids, comprise at least a portion of the C- or D-region from IGF-1, and inhibit IGF-1-induced autophosphorylation of IGF-1 receptors.--

The paragraph starting at page 12, line 11 has been amended as follows:

-- The invention further provides a method of identifying indirect agonists of IGF-1 comprising co-crystallizing a candidate indirect agonist of IGF-1 with IGF-1 to form a co-crystalline structure and determining if the candidate agonist binds to one or both of two patches on IGF-1 (SEQ ID NO: 1), wherein one patch has the amino acid residues Glu 3, Thr 4, Leu 5, Asp 12, Ala 13, Phe 16, Val 17, Cys 47, Ser 51, Cys 52, Asp 53, Leu 54, and Leu 57, and the second patch has the amino acid residues Val 11, Gln 15, Phe 23, Phe 25, Asn 26, Val 44, Phe 49, and Arg 55, and wherein binding occurs if there is at least one contact between each listed amino acid residue of a given patch and the candidate agonist that is less than or equal to 6 angstroms in the co-crystalline structure. In preferred embodiments, the candidate agonist inhibits binding of IGFBP-1 or -3 to IGF-1 (SEQ ID NO: 1) at least as well as N, N-bis(3-D-gluconamidopropyl)-deoxycholamine. More preferred is the method wherein inhibition of binding is measured using a competition assay between N, N-bis(3-D-gluconamidopropyl)-deoxycholamine

and the candidate agonist. Most preferred is the method wherein inhibition of binding is measured by pre-incubating N, N-bis(3-D-gluconamidopropyl)-deoxycholamine or the candidate agonist with IGF-1 expressed on bacteriophage particles and measuring residual binding of IGF-1 to IGFBP-1 or IGFBP-3 in a plate-based ELISA assay.--

The paragraph starting at page 10, line 8 has been amended as follows:

-- Figures 7A and 7B are ribbon diagrams of IGF-1 (SEQ ID NO: 1) demonstrating, as does Fig. 4, that the detergent used in the reservoir solution (N, N-bis(3-D-gluconamidopropyl)-deoxycholamine), shown in stick form, binds into a small hydrophilic cleft at the base of the B-helix. In Fig. 7A the detergent head group is inserted into the cleft lined by residues Leu 5, Phe 16, Val 17, Leu 54, and Leu 57 of SEQ ID NO: 1. The various shades of gray are according to the alanine-scanning mutagenesis results of Dubaquie and Lowman, *supra*, with the Phe 16, Val 17, and Leu 5 regions indicating a 5-10 fold reduction, the Glu 3 region a 10-100 fold reduction, and the Pro 63 and Pro 63' regions a >100 fold reduction in affinity for IGFBP-1, where the amino acid residues are numbered with reference to the amino acid sequence of IGF-I (SEQ ID NO: 1). The black part at the far right corresponds to the symmetry-related IGF-1 molecule that forms the crystallographic dimer. The circle near Leu 54 indicates the C10 atom of the detergent, which differs from another detergent (3-((3-cholamidopropyl) dimethylammonio)-1-propane sulphonate; or CHAPS) by having a hydroxyl group at this position. Fig. 7B shows the view from the opposite surface of the detergent and depicts the interactions of the detergent molecule with a symmetry-related IGF-1 molecule. As in Fig. 7A the various shades of gray are according to the alanine-scanning mutagenesis results of Dubaquie and Lowman, *supra*, with the group near Gln 15 indicating a 5-10 fold reduction, the far left medium gray molecules, the Leu 10 region molecules, and the far right medium gray region indicating a 10-100 fold reduction, and the black regions at Phe 49 and Gly 7 indicating a >100 fold reduction in affinity for IGFBP-1. The black

regions to the right of the detergent molecule correspond to the symmetry-related IGF-1 molecule that forms the crystallographic dimer. The circle near Gln 15 indicates the C10 atom of the detergent, as noted above for Fig. 7A. This figure was prepared using the program INSIGHT (MSI, San Diego, CA).--

The paragraph starting at page 23, line 15 has been amended as follows:

-- The invention further provides a method of identifying indirect agonists of IGF-1 comprising co-crystallizing the candidate agonist with IGF-1 to form a co-crystalline structure and determining if the candidate agonist molecule binds to one or both of two patches on IGF-1. The first patch contains the amino acid residues Glu 3, Thr 4, Leu 5, Asp 12, Ala 13, Phe 16, Val 17, Cys 47, Ser 51, Cys 52, Asp 53, Leu 54, and Leu 57, and the second patch contains the amino acid residues Val 11, Gln 15, Phe 23, Phe 25, Asn 26, Val 44, Phe 49, and Arg 55 of IGF-I (SEQ ID NO: 1). For purposes herein, binding means that there is at least one contact between each listed amino acid residue of a given patch and the candidate agonist molecule that is less than or equal to 6 angstroms in the co-crystalline structure. Such a candidate agonist molecule will have the property of inhibiting binding of IGFBP-1 or IGFBP-3 to IGF-1. The preferred such candidate agonist molecule will inhibit binding of IGFBP-1 or -3 to IGF-1 at least as well as N, N-bis(3-D-gluconamidopropyl)-deoxycholamine. More preferred is the method wherein inhibition of binding is measured using a competition assay between N, N-bis(3-D-gluconamidopropyl)-deoxycholamine and the candidate agonist molecule. Most preferred is the method wherein inhibition of binding is measured by pre-incubating N, N-bis(3-D-gluconamidopropyl)-deoxycholamine or the candidate agonist molecule with IGF-1 expressed on bacteriophage particles and measuring residual binding of IGF-1 to IGFBP-1 or IGFBP-3 in a plate-based ELISA assay. --

The paragraph starting at page 28, line 8 has been amended as follows:

-- After several cycles of model building and phase combination, the final model, shown in Figure 2, contains residues 3-34 and 41-64 of IGF-1 (SEQ ID NO: 1), a single-bound detergent molecule, and 46 water molecules. The R factor to 1.8 Å is 23.7%, and the free R factor is 26.9%, with good stereochemistry. The N-terminal B-region corresponds to residues 3-28, the C-region from 29-34, a stretch of poorly ordered residues from 35-40, and the A-region from 42-62 of IGF-I (SEQ ID NO: 1). The D-region (63-70) is essentially disordered.--

The text between page 29, line 2 and page 31, line 10 has been amended as follows:

-- IGF-1 (SEQ ID NO: 1) is composed primarily of three helical segments corresponding to the B-helix (IGF-1 residues 7-18) and two A-helices (IGF-1 residues 43-47 and 54-58) of insulin. The hydrophobic core is essentially identical to that described for the NMR structures of IGF-1, including the three disulfide linkages between Cys 6 and Cys 48, Cys 18 and Cys 61, and Cys 47 and Cys 52, as noted in the references above. Residues 3 through 6 do not form any regular secondary structure, and hence, the structure described herein can be classified as being most similar to the T-form of insulin (Derewenda *et al.*, Nature, 338: 594-596 (1989)). Indeed, when IGF-1 and the T-form of insulin are superimposed on the Cα positions of their respective helical segments (IGF-1 SEQ ID NO: 1) residues 8-19, 42-49, and 54-61; insulin (SEQ ID NO: 3) residues B9-B20, A1-A8, and A13-A20) the RMSD is only 0.47 angstroms. As in insulin, residues 18-21 at the end of the B-helix form a type II' β-turn, which redirects the backbone from the B-helix into an extended region. Residues 24-27 form a type VIII ß-turn to accommodate the C-region, which extends away from the core of IGF-1, and interacts with a symmetry-related molecule. Residues 30-33 form a well-defined type II beta-turn, prominently displaying Tyr 31 at the i+1 position. Residues 35-40 have not been

modeled, as the electron density in this region is weak and disconnected. Only the first two residues of the D-region (residues 63 and 64) are ordered in the structure.

The C-region of IGF-1 (SEQ ID NO: 1) mediates a two-fold symmetric crystal-packing interaction across the *a*-axis of the unit cell. This interaction buries 689 $Å^2$ of solvent-accessible surface area from each molecule of IGF-1, or 1378 $Å^2$ total, and is the largest interface in the crystal. A total of 28 intermolecular contacts of distance 3.6 Å or less are formed via this interface, with the next most extensive crystal packing interaction forming only nine contacts. The core of the interface is dominated by Tyr24 and Pro28 from each monomer, which bury 39 $Å^2$ and 57 $Å^2$ of solvent-accessible surface area, respectively. The aromatic ring of Tyr 31, which lies at the tip of the loop at the furthest point from the core of IGF-1, packs against the phenolic rings of Phe 23 and Phe 25 of the symmetry-related molecule. In addition to these hydrophobic interactions, two main-chain hydrogen bonds (Tyr 31 N-Phe 23 O and Ser 34 N-Asp 20 O of IGF-I, SEQ ID NO: 1) are present in the dimer interface. Residues from the D-region (62-64) are also partially sequestered by this dimer formation. Because of these interactions, most of the C-region in the crystal is well-ordered, providing the first high-resolution view of the conformation of this biologically important loop.

Although 72 detergent compounds, including the similar 3-((3-cholamidopropyl) dimethylammonio)-1-propane sulphonate (CHAPS) and 3-((3-cholamidopropyl)dimethylammonio)-2-hydroxypropanesulfonic acid (CHAPSO) detergents, were screened in crystallization trials, only N, N-bis(3-D-gluconamidopropyl)-deoxycholamine yielded crystals. A single molecule of N, N-bis(3-D-gluconamidopropyl)-deoxycholamine interacts with residues, forming a small hydrophobic cleft on one surface of IGF-1 (SEQ ID NO: 1) (Leu 5, Phe 16, Val 17, Leu 54, and Leu 57) (Fig. 7A). The preference for N, N-bis(3-D-gluconamidopropyl)-deoxycholamine is explained, without being limited to any one theory, by the absence of an oxygen atom at position C10 in the detergent molecule. This region of the detergent is

in close contact with the side chain atoms of residues Leu 5, Leu 54, and Leu 57 in IGF-1. The opposite face of the detergent mediates a symmetry contact with residues Val 11, Leu 14, and Gln 15 of a symmetry-related IGF-1 molecule. Intriguingly, this face of N, N-bis(3-D-gluconamidopropyl)-deoxycholamine also contacts the edge of the dimer interface, with close contacts to Phe 23 and Phe 25 of the same IGF-1 molecule, as well as Tyr 31 and Gly 32 of the dimeric partner (Fig. 7B). A more detailed analysis indicates that the detergent binds to two patches of binding pockets of IGF-1 (SEQ ID NO: 1). One patch has the amino acid residues Glu 3, Thr 4, Leu 5, Asp 12, Ala 13, Phe 16, Val 17, Cys 47, Ser 51, Cys 52, Asp 53, Leu 54, and Leu 57, and the second patch has the amino acid residues Val 11, Gln 15, Phe 23, Phe 25, Asn 26, Val 44, Phe 49, and Arg 55. Binding is defined by having at least one contact between each listed amino acid residue and the candidate agonist molecule that is less than or equal to 6 angstroms.

Discussion

The C-region in the IGF-1 crystal structure extends out from the core of the molecule, with residues 30-33 forming a canonical type II beta-turn, and the remainder of the C-region forming a crystallographic dimer with a symmetry-related molecule. Tyr 31 has been implicated as being a critical determinant for IGF-1R binding (Bayne *et al.* (Vol. 264), *supra*; Bayne *et al.* (Vol. 265), *supra*; Cascieri et al., supra), and its location at the tip of this extension places it in an ideal location to interact with a receptor molecule. While this region of IGF-1 is not well-defined by NMR data, the conformation of the C-region in the crystal is likely to reflect a prevalent solution conformation. There is evidence of a reverse turn at the tip of the loop and a hinge bending at the loop termini of IGF-2 (SEQ ID NO: 2; Torres *et al., supra*). Thus, while crystal packing forces undoubtedly help stabilize the orientation of this loop, its conformation appears to be consistent with the solution structure of the closely related IGF-2.

The size of the interface formed by the crystallographic dimer is well within the range of buried surface area in known biological complexes (Janin and Chothia, J. Biol. Chem., 264: 16027-16030 (1990)). In addition, this interaction partially excludes from solvent several of the residues known to be important for binding to the IGF-1R, including Phe 23 (69% buried), Tyr 24 (64% buried), Phe 25 (29% buried), and Tyr 31 (38% buried) of IGF-I, SEQ ID NO: 1. Other groups have also reported homodimeric interactions of IGF-1 (SEQ ID NO: 1) and IGF-2 (SEQ ID NO: 2). Laajoki *et al.*, (2000), *supra*, report that at a concentration of 1 mM, an engineered form of IGF-1 (Long-[Arg^3]IGF-1) partitions into about 20% dimer/80% monomer, a ratio that is in good agreement with the estimate of 3.6 mM K_d. In their NMR study of IGF-2, Torres *et al.*, *supra,* reported that the amide protons of residues in the C-region were slowly exchanging with solvent, suggesting that IGF-2 forms a homodimer in solution. However, despite the significant amount of surface area that is buried upon dimer formation in the crystal, the affinity of IGF-1 for itself is very weak. In addition, the known binding stoichiometry of one IGF-1 molecule per receptor dimer (De Meyts, *supra*) makes it difficult to rationalize the biological significance of IGF-1 dimerization. In conclusion, the IGF-1 dimer in this crystal form results from the high concentration of IGF-1 in the crystallization experiment, and does not represent a physiologically relevant form of the molecule.

The very low quality of NMR spectroscopic data obtained for IGF-1 at near-neutral pH has been attributed to a combination of self-association and internal mobility that leads to a large variation in resonance line width (Cooke *et al., supra*). As a result, NOESY spectra acquired on IGF-1 contain many broad, overlapped peaks and few sharp well-resolved correlations. NOESY spectra collected for IGF-1 in the presence of an excess of N, N-bis(3-D-gluconamidopropyl)-deoxycholamine have a similar appearance. Thus, detergent binding is not sufficient to eliminate the aggregation or inherent flexibility of IGF-1 and does not facilitate characterization of the solution conformation

of the protein. Likewise, detergent binding does not alter the aggregation state of IGF-1, as assessed by analytical ultracentrifugation experiments in the presence of N, N-bis(3-D-gluconamidopropyl)-deoxycholamine. This is in contrast to observations in the crystalline state where addition of N, N-bis(3-D-gluconamidopropyl)-deoxycholamine leads to a well-packed crystallographic dimer and crystals that diffract to high resolution. Jansson *et al.*, J. Biol. Chem., 273: 24701-24707 (1998) noted that the lack of NMR assignments in the region immediately surrounding Cys 6 of IGF-I, SEQ ID NO: 1, which includes Leu 5 and Gly 7, was indicative of the Cys 6-Cys 48 disulfide undergoing intermediate exchange between a cis and trans configuration. The fact that the detergent binds to one face of the B-helix immediately opposite this disulfide suggests, without being limited to any one theory, that it may serve to stabilize this region of the molecule by more complete packing of the hydrophobic cleft. Indeed, in the crystal structure herein, the Cys 6-Cys 48 is clearly in the trans conformation, and there is no evidence of multiple conformations.--

The paragraph starting at page 33, line 36 has been amended as follows:

-- Several studies have identified residues in IGF-1 (SEQ ID NO: 1) that are important for IGFBP binding (Clemmons *et al.*, Endocrinology, 131: 890-895 (1992); Dubaquie and Lowman, *supra*; Jansson *et al.*, *supra*; Oh *et al.*, (1993), *supra*; Lowman *et al.*, (1998), *supra*; and Dubaquie *et al.*, Endocrinology, 142: 165-173 (2001)). Dubaquie and Lowman, *supra,* identified two distinct patches on IGF-1 that interact with IGFBP-1 and IGFBP-3. Patch I consists of Glu 7, Leu 10, Val 11, Leu 14, Phe 25, Ile 43, and Val 44, while patch 2 consists of Glu 3, Thr 4, Leu 5, Phe 16, Val 17, and Leu 54. In the crystal structure of IGF-1, these two patches are involved in detergent-mediated crystal packing contacts. (Specifically, Patch 1 of the crystal structure of IGF-1 consists of amino acid residues Glu 3, Thr 4, Leu 5, Asp 12, Ala 13, Phe 16, Val 17, Cys 47, Ser 51, Cys 52, Asp 53, Leu 54, and Leu 57, and Patch 2 of the crystal structure of IGF-1 consists

of amino acid residues Val 11, Gln 15, Phe 23, Phe 25, Asn 26, Val 44, Phe 49, and Arg 55, wherein binding occurs if there is at least one contact between each listed amino acid residue and the candidate agonist molecule that is less than or equal to 6 angstroms.)--

The paragraph starting at page 35, line 1 has been amended as follows:

-- In total, 899 distance restraints (779 intra-IGF-1; 33 intra-peptide; 87 intermolecular), 16 hydrogen bond restraints in helix I, and 138 dihedral angle restraints (71 Φ; 44 ψ; 23 $\chi 1$) were used to generate an ensemble of structures using a torsion-angle dynamics protocol with the computer program CNX (Accelrys Inc., San Diego). The structure of IGF-1 (SEQ ID NO: 1) was well defined for the B-region (residues 2 – 25) and the A-region (residues 41-63) with a mean RMSD from the mean structure for backbone heavy atoms of 0.32 ± 0.06 Å. The C-region (26-40) and the D-region (62-70) were not well defined by the available data. The 20 structures of lowest restraint violation energy had good backbone stereochemistry (80% of residues in the most favored region of Φ/ψ space with none in disallowed regions) and contained few violations of the experimental restraints (mean maximum distance restraint violation 0.09 ± 0.02 Å). IGF-F1-1 adopts a conformation very similar to that determined for the peptide by itself in solution. The conformation of IGF-1 contains three helices (residues 7-18, 43-49, and 54-60) and is similar to that seen at lower resolution in previous NMR studies of uncomplexed IGF-1 (see *e.g.* Cooke *et al., supra*; Sato *et al., supra*; and Laajoki *et al., supra*).--